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Gbb/BMP signaling is required to maintain energy homeostasis in *Drosophila*

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ABSTRACT

The coordination of animal growth and development requires adequate nutrients. During times of insufficient food, developmental progression is slowed and stored energy is utilized to ensure that cell and tissue survival are maintained. Here, we report our finding that the Gbb/BMP signaling pathway, known to play an important role in many developmental processes in both vertebrates and invertebrates, is critical in the Drosophila larval fat body for regulating energy homeostasis. Animals with mutations in the Drosophila BMP-5,7 orthologue, *glass bottom boat* (*gbb*), or in its signaling components, display phenotypes similar to nutrient-deprived and *Tor* mutant larvae. These phenotypes include a developmental delay with reduced overall growth, a transparent appearance, and altered total lipid, glucose and trehalose levels. We find that Gbb/BMP signaling is required in the larval fat body for maintaining proper metabolism, yet interestingly, following nutrient deprivation larvae in turn show a loss of BMP signaling in fat body cells indicating that Gbb/BMP signaling is a central player in homeostasis. Finally, despite strong phenotypic similarities between nutrient-compromised animals and *gbb* mutants, distinct differences are observed in the expression of a group of starvation responsive genes. Overall, our results implicate Gbb/BMP signaling as a new pathway critical for positive regulation of nutrient storage and energy homeostasis during development.

Introduction

Bone morphogenetic proteins (BMPs) belong to the transforming growth factor-β (TGF-β) superfamily of signaling molecules. These superfamily members generate secreted bioactive ligands that regulate critical cellular processes during the development of both vertebrates and invertebrates (Chen et al., 2004; Herpin et al., 2004; Hogan, 1996; Nakayama et al., 2000). In Drosophila, the vertebrate BMP2,4, and BMP5,6,7,8 orthologues are encoded by *decapentaplegic* (*dpp*) and *glass bottom boat* (*gbb*) genes, respectively. These two BMP ligands have pleiotropic functions during Drosophila development with overlapping roles in regulating growth and patterning of the wing imaginal disc, as well as non-overlapping roles in the specification of embryonic dorsoventral cell fates and retrograde signaling at the larval neuromuscular junction (Chen et al., 2004; Herpin et al., 2004; Marques, 2005; Nakayama et al., 2000; O'Connor et al., 2006; Ray and Wharton, 2001).

gbb originally received its name due to the profound transparency of third instar larvae null for *gbb* function (Khalsa et al., 1998). Larval transparency is a phenotype long known to be associated with extended periods of nutrient deprivation (Britton and Edgar, 1998; Hadorn, 1951). Like starved larvae, we find that the transparency of *gbb* mutant larvae is largely due to a decrease in opacity of the larval fat body, a functional equivalent of the white adipose tissue and liver of vertebrates (Arrese et al., 2001; Britton and Edgar, 1998; Canavoso et al., 2001). Our observation of this notable phenotypic resemblance raised the possibility that a defect in Gbb signaling could impact the nutritional status of developing larvae, and we set out to investigate this possibility.

The ability of organisms to properly obtain, store, and metabolize nutrients is essential for their growth and development. When food is plentiful, sugars are stored as glycogen in the muscles and liver of vertebrates, with fats, or lipids, stored in the form of triacylglycerides (TAGs), in the liver and adipose tissue. Both fats and sugars are similarly stored in the insect liver and adipose organ equivalent, the fat body (Arrese et al., 2001; Canavoso et al., 2001) with sugar (trehalose) levels regulated by insulin and insect glucagon (adipokinetic hormone, AKH) secreting neurosecretory cells (Leopold and Perrimon, 2007). In the absence of adequate nutrient sources, animals will mobilize their internal nutrient stores in order to utilize their energy and ensure survival (Finn and Dice, 2006; Wigglesworth, 1972). TAGs are mobilized first to provide energy for high demand tissues, followed by the mobilization of glycogen and protein stores (Arrese et al., 2001; Finn and Dice, 2006). Under severe nutrient deprivation, not only are energy stores depleted but the time to complete development and reach maturity is significantly lengthened and the overall size of the developing animal can be dramatically reduced (Edgar, 2006; Mirth and Riddiford, 2007). The developmental pathways and molecular mechanisms involved in sensing available nutrients, as well as those involved in the regulation of lipid metabolism and energy homeostasis during the development are

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coming to light as is their conservation between invertebrates and vertebrates.

Mis-regulation of factors responsible for coordinating nutrient storage and metabolism in mammals increases the propensity of developing metabolic disorders, such as obesity and diabetes (Fingar and Blenis, 2004; Oldham and Hafen, 2003; Wullschleger et al., 2006). In both vertebrates and invertebrates, the insulin/IGF and Target of Rapamycin (TOR) signaling pathways have been implicated in the regulation of nutrient uptake, storage, and metabolism. Insulin signaling regulates sugar levels and stimulates nutrient storage in both Drosophila and vertebrates, while a reduction in insulin signaling results in the breakdown of stored nutrients (Britton et al., 2002; Saltiel and Kahn, 2001). TOR kinase activity is influenced by amino acid availability, and decreases in TOR function have been shown to result in reduced lipid stores in both Drosophila and vertebrates (Gao et al., 2002; Hara et al., 1998; Luong et al., 2006; Radimerski et al., 2002; Stocker et al., 2003; Um et al., 2004). Consistent with these findings *Tor* mutant Drosophila larvae show a reduction in size and a transparent larval phenotype that has been attributed to the utilization of stored energy (Hennig and Neufeld, 2002; Zhang et al., 2000). Thus, as is true of insulin signaling, TOR responds to the nutritional state of both mammals and Drosophila and plays a key role in the balance of energy stores.

Recent studies have shown that many of the pathways critical for metabolism are conserved between vertebrates and insects (Canavoso et al., 2001; Garofalo, 2002; Oldham and Hafen, 2003; Pan et al., 2004). This conservation and the tractable nature of *Drosophila melanogaster* genetics provide an ideal model system to identify key regulators of nutrient sensing, energy storage, and metabolism. Central to sustaining proper energy homeostasis during development is the larval fat body, as it coordinates the function and growth of multiple tissues with the energy demands of the organism. For example, a reduction in the ability of the fat body to sense amino acids leads to a reduction in both cell growth and cell proliferation of other tissues, such as the larval salivary glands and imaginal discs (Colombani et al., 2003). Furthermore, the fat body is in intimate communication with tissues producing hormonal signals, such as the brain and ring gland, which are critical for maintaining a stable equilibrium between energy storage and utilization (Edgar, 2006; Mirth and Riddiford, 2007).

In Drosophila larvae, TAGs from ingested foodstuffs are broken down in the midgut by digestive lipases, transported across the midgut endodermal epithelium into the hemolymph, and ultimately stored in vesicles, or lipid droplets within the fat body (Arrese et al., 2001). These stores are utilized as the primary energy source for the high demands of metamorphosis. However, during periods of food scarcity, stored TAGs can be metabolized prior to metamorphosis through the hormonal stimulation of lipases (Beenakkers et al., 1985). Mobilized lipids are ultimately transported to various target tissues, where the energy released by β-oxidation of fatty acids allows for continued growth and organismal survival (Arrese et al., 2001; Canavoso et al., 2001). A recent study has implicated the involvement of a cluster of hepatocyte-like cells, the larval oenocytes, in lipid mobilization by demonstrating that during periods of fasting, oenocytes accumulate lipids through a process that is dependent upon the appropriate metabolic functioning of the fat body (Gutierrez et al., 2007).

Based on the striking phenotypic similarity between *gbb* mutants and nutrient deprived larvae, we investigated the possibility that BMP signaling is important in regulating the nutritional status of Drosophila during larval development. Here, we report that the Gbb/BMP signaling pathway plays a critical role in regulating metabolism. Gbb/BMP signaling occurs in fat body cells and affects metabolic stores such that nutritional status and growth are properly achieved during the larval stages with the appropriate developmental progression.

Materials and methods

Drosophila melanogaster strains

Flies were raised at 25 °C on standard cornmeal/sugar/agar food. Oregon R flies were used as wild-type control in all experiments. Alleles are described in Flybase (www.flybase.net) except for the hypomorphic Tor^{15} allele obtained from Sean Oldham. The following fly stocks were used for Gal4-UAS experiments: *UASgbb9.9* (Khalsa et al., 1998), *FB-Gal4* (Gronke et al., 2003), *BO-Gal4* (Gutierrez et al., 2007), *ppl-Gal4* (Colombani et al., 2003). The expression of *FB-Gal4* and *ppl-Gal4* is most prominent in the larval fat body with lower levels of expression in the oenocytes (FB-Gal4) or in the brain and gut (ppl-Gal4). *BO-Gal4* expression is high in embryonic and early first instar larval oenocytes but is undetectable in these cells during later larval stages. For *UAS-dad* FLP-out clones, *hsFLP; UAS-dad* flies were crossed to *AyGal4 UASnGFP* flies and allowed to lay on an apple juice plate for 6 h. First instar larvae were collected 0–2 h after hatching and placed in a 37 °C water bath for 10–15 min to induce UAS-dad, UAS-nGFP expressing clones. These larvae were then immediately placed on Bo-C_{12} containing food until dissection as crawling third instar larvae.

Larval and fat body analysis

Images of wandering third instar larvae were taken on a Leica MZ FLIII stereomicroscope using the identical settings. For WT nutrient-deprived larval images, mid-third instar larvae (96 h after hatching) were placed in an empty vial on filter paper soaked in PBS and imaged 12–24 h later. Fat bodies were dissected from equivalently staged third instar larvae just prior to wall crawling in 1xPBT, immediately fixed in 4% paraformaldehyde, pH 7 for 10 min and then incubated with phalloidin for 20 min. Fat bodies were mounted in the same volume of 50% glycerol with cover glass spacers to ensure that samples were not compressed. For fat body cell size measurements, 579 wild-type Oregon R cells and 457 gbb^{1} cells from two regions: the anterior lobe and the posterior section just anterior to the larval gonad were measured from multiple fat bodies. The cell circumference was calculated using NIH Image.

Bo-C_{12} feeding

No more than 30 first instar larvae (0–4 h after hatching) were placed in 0.4 g food mixed with 200 μl of 5 μM Bo-C_{12} (4,4-difluoro-5-methyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoic acid (BODIPY® 500/510 C1, C12) Molecular Probes #D-3823). Larvae were raised at 25 °C in the dark. Wandering third instar larval fat bodies were dissected in PBS and fixed in 4% paraformaldehyde for 5–7 min and stained with 1 μg/ml phalloidin-TRITC (Sigma P1951) and Hoechst (1:1000 Molecular Probes H3570) for 15 min. Fat bodies were mounted in 80% glycerol, 0.5% n-propyl-galate in PBS. Fluorescent images were obtained using a LeicaTCS-SP2-AOBS confocal microscope. All confocal images within an experiment were obtained under the same settings. The absolute level of Bo-C_{12} fluorescence present in fat body lipid droplets of the same genotype could vary slightly; however, differences between different genotypes or treatments were consistent from experiment to experiment, where nutrient-deprived larvae and BMP mutant fat bodies exhibited higher levels of fluorescence when compared to those from wild-type fed larvae. VOX and DeskVox (http://www.calit2.net/~jschulze/projects/vox/) were used to analyze confocal Z-series/3D data sets.

Immunohistochemistry

Fat bodies of crawling third instar larvae were dissected and fixed in 4% formaldehyde for 10 min, blocked in 1% Normal Goat Serum (NGS) and 0.1% Triton-X in PBS for 1 h. Primary antibody (anti-PS1 1:1000)

(Persson et al., 1998) was added and tissue was incubated at room temperature for 2 h. Secondary antibody (Alexa 568) was used at 1:3000. Fat bodies were stained with Hoechst (1:1000) and mounted in 80% glycerol and 0.5% N-propyl-galate in PBS. Images were taken using the same settings with a Zeiss 510 confocal laser scanning microscope.

Wing disc area

Female larvae raised at 25 °C were dissected in PBS and wing imaginal discs fixed for 20 min in 4% paraformaldehyde/PBS. Discs were then stained with 1 μg/ml Phalloidin-TRITC (Sigma P1951) for 20 min at room temperature. Area was determined using Image J (http://rsbweb.nih.gov/ij/). Wing disc area was traced three times, each time determining the area in pixels, and an average for each wing disc was calculated. 10–12 wing discs were examined for each genotype. For wild-type starved larval conditions, the larvae were placed on PBS soaked filter paper for 12 h prior to pupariation.

Oenocyte analysis

Crawling third instar larvae (either fed or starved up to 24 h before crawling) were dissected in PBS and stained with Oil Red O according to Gutierrez et al., 2007. Immunohistochemistry was carried out as for larval fat bodies (above), except the body walls of dissected third instar larvae were fixed for 20 min. Tissues were mounted in 80% glycerol, 0.5% n-propyl-galate, and images were taken using the same settings on a Zeiss Axiovert 200M light microscope.

Construction of UASgbbRNAi

A ~400 bp region of *gbb* with little to no homology with other sequences in the genome was amplified by PCR using the following forward and reverse primers: 5′GAGAGATCTCGAACTGCGTCACG3′ and 5′CATGGAATTCGTCGTCCAGCTTG3′, and cloned into the *Bgl*II and *Eco*RI sites of SympUAST-w. Multiple transgenic lines were generated and tested for their ability to phenocopy *gbb* loss of function when crossed to various Gal4 lines.

RT-PCR analysis

mRNA was isolated using RNAeasy kit (Qiagen 74104) from wild type or mutant early third instar or second instar larvae that had been fed or starved for 12 h after the third instar or 4 h after the second instar molt, respectively. cDNA was produced by oligo dT and MLV-reverse transcriptase (Sigma). The cycling profile was optimized for each gene primer set and for amplification within the logarithmic phase (see also Zinke et al., 2002). All primers (Supplemental Table 2) span an intron(s) except *gbb*, which does not have an intron. PCR products from at least 2 separate RNA samples were run on 0.8% agarose gel, which was stained with Vistra Green (Amersham RPN5786 1:10,000) for 1 h. Gels were scanned on a Typhoon 9410, and ImageQuant software was used to determine the intensity of each band. The ratio of intensities of bands for the gene of interest to *actin* was then determined in each sample.

TAG analysis

Lipids were isolated from 10 wandering third instar larvae using an assay modified from Bligh and Dyer (1959). Lipids were spotted on a Silica chromatography plate (Baker Si250, Mallinckrodt Chemicals) and resolved in a chamber lined with Whatman paper and in heptane: isopropyl ether:acetic acid (60:40:4) using lipid standards (1787-1AMP Sigma). TLC plate was allowed to dry and placed in a chamber with iodide crystals for at least 1 h and immediately scanned. ImageQuant software was used to analyze intensity and area of bands for TAGs. TAG amount was normalized for weight of the larvae, and each genotype was compared to the TAG levels of normally fed wild-type larvae.

GC-MS

Lipids were isolated according to Kunte et al. 2006 using a known amount of C_{15} as an internal control. To yield fatty acid methyl esters, the lipid extract was dried under helium and incubated at 50 °C with 50 μl of benzene, 50 μl of methanol, and 5 drops of trimethylsilyldiazomethane (Sigma 362832). GC-MS analysis was performed using a JEOL JMS-600 mass spectrometer operated in the electron impact ionization mode. The GC was equipped with a 30-m HP-5MS column. Amount of each fatty acid class was compared to C_{15}, and total lipid was compared to C_{15} relative to the wild-type fed larvae sample.

Statistics

Data presented are mean ± SEM. The Student's *t*-test was used for comparisons between 2 groups. Significance was assumed for p values <0.05 unless otherwise noted.

Results

gbb mutant larvae exhibit growth defects

gbb mutants progress through development at a slower rate than wild-type larvae and *gbb* null animals die prior to pupation (Wharton et al., 1999). During the third larval instar, *gbb* mutants appear morphologically distinct from their wild-type counterparts. At equivalent developmental times during the third instar, *gbb* mutant larvae are slightly smaller than wild-type larvae but exhibit a markedly transparent appearance (Figs. 1A, D). This transparency is primarily due to a change in opacity of the fat body. All regions of the fat body are present in *gbb* mutant larvae, however, the size of individual cells is reduced (Fig. 1B) such that the entire organ is somewhat smaller in overall size. In addition to the smaller size and the dramatic transparency of *gbb* mutant fat bodies, the intracellular lipid droplets in mutant fat body cells are more variable in size than that seen in wild type fat bodies, with mutant cells often having only 2–4 very large lipid droplets per cell (Figs. 1B, C). A change in lipid droplet size is often associated with a change in lipid metabolism (Brasaemle, 2007) and could be an indication that in *gbb* mutants, the metabolism of lipids, or metabolism in general, is in some way altered.

We had previously observed a general reduction in the size of *gbb* mutant larval organs, such as the brain and imaginal discs (Khalsa et al., 1998). In some tissues such as the larval salivary glands where it is possible to count all cells, we have noted that gbb^1 mutants have a 14 ± 3 % reduction in cell number ($n = 28$). Here, we document that late third instar *gbb* mutant wing imaginal discs are in fact 50% smaller in overall area compared to the area of wild-type wing discs of the same developmental stage (Fig. 1E). These findings are consistent with a requirement for *gbb* in ensuring normal growth of cells and tissues. Like *gbb* mutants, larvae mutant for genes encoding other BMP signaling components, *sax* (a type I receptor) and *Mad* (encoding the R-Smad) also show larval transparency and a reduction in organ size (Fig. S1).

Loss of Gbb signaling leads to a reduction in metabolic stores

Larval transparency, reduced cell and tissue growth, and a change in lipid droplet size are all phenotypes strikingly similar to those observed in wild-type larvae that have been deprived of nutrients (Britton and Edgar, 1998) (Figs. 1D, E; Figure S1; data not shown). These same phenotypes have also been reported in animals that lack the activity of the TOR (target of rapamycin) kinase, known to play an important role in the regulation and sensing of nutrient stores within

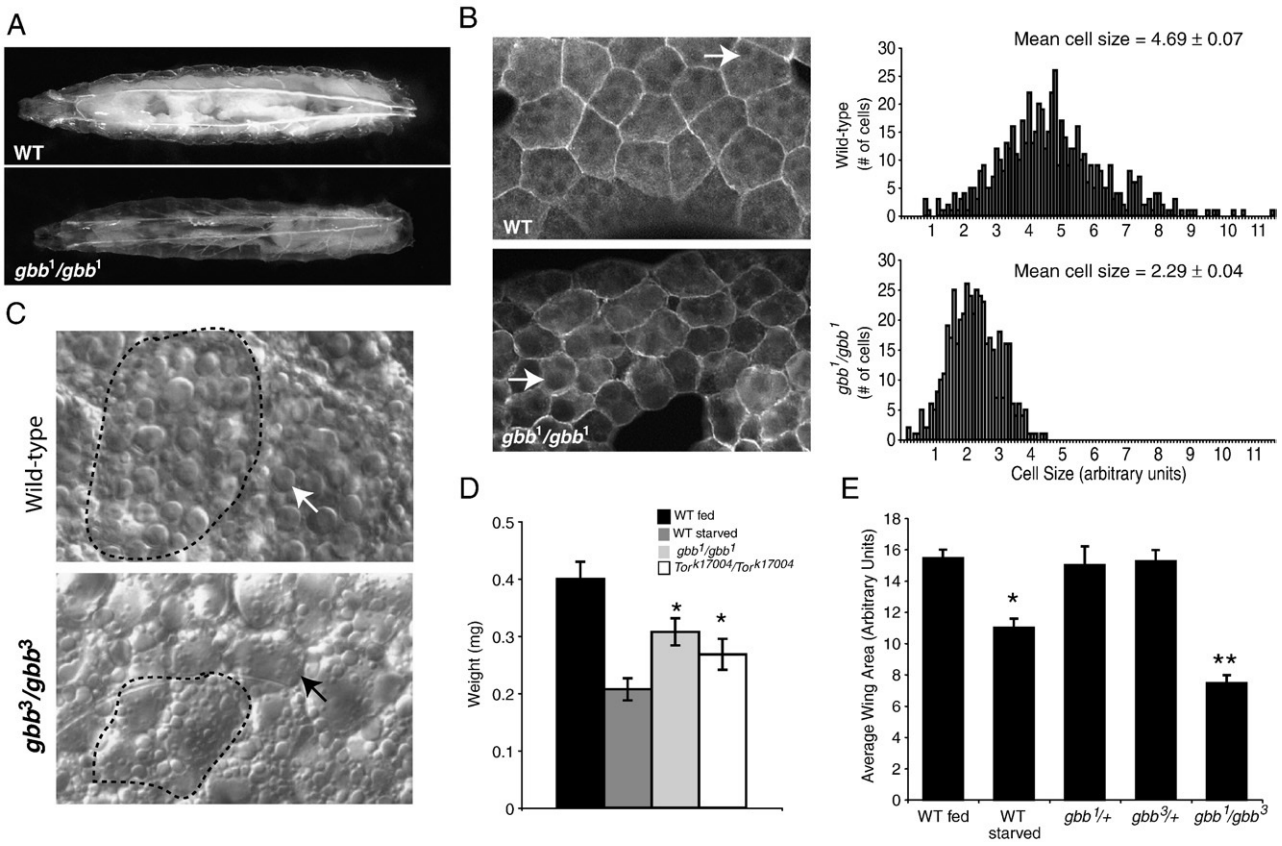

Fig. 1. *gbb* mutants exhibit abnormal growth and altered fat body morphology. (A) *gbb* null larvae (gbb^1/gbb^1) are transparent and slightly smaller compared to wild-type larvae of the same developmental stage. (B) (left panels) Phalloidin staining of dissected fat bodies reveals a difference in the size of fat body cells between wild-type and *gbb* mutants. (Right panels) The distribution of fat body cell sizes is shown for third instar wild-type and *gbb* null larvae, where the average cell size is approximately 50% smaller than wild-type fat bodies ($n>450$). (C) High magnification Nomarski images of dissected wild type and *gbb* mutant fat bodies. Variation in the size and distribution of lipid droplets (arrow) is apparent in *gbb* mutant tissue. Cell out line–dotted line. (D) The body mass of third instar larvae is reduced for *gbb* and *Tor* mutants, as well as for wild type larvae that have been deprived of nutrients. (E) The size of crawling third instar larval wing imaginal discs is approximately 30% smaller in wild-type larvae deprived of nutrients for 12 h compared with discs from fed wild-type larvae. Wing discs from crawling gbb^1/gbb^3 larvae are 50% reduced in size compared with control discs. ($n>10$) * and **$p<0.0001$ compared with wild-type fed and control discs, respectively (Student's *t*-test).

the Drosophila fat body (Britton and Edgar, 1998; Butterworth et al., 1965; Colombani et al., 2003; Zhang et al., 2000) (Fig. 2B; data not shown). The similarity of gbb mutant larval phenotypes with those of nutrient deprived wild type larvae and *Tor* mutant larvae led us to investigate the status of nutrient stores in *gbb* mutant larvae.

In insects, triacylglycerides (TAGs) stored in fat body lipid droplets are the main form of stored energy (Arrese et al., 2001; Canavoso et al., 2001). Protein and glycogen deposits are also found in the fat body and the major form of circulating carbohydrates are glucose and trehalose. During periods of inadequate food, the lipids and sugars are the first stored reserves to be mobilized to ensure that there is continued growth and progression through development (Finn and Dice, 2006; Wigglesworth, 1972). We did not find a significant difference in overall protein levels between wild-type and *gbb* mutant third instar larvae when normalized for body mass (Fig. 2D). However, we found that both lipids and sugars were reduced in *gbb* mutants (Figs. 2A–C). We also assayed for the level of nutrient stores in wild-type larvae that had been deprived of nutrients for 12 h and in *Tor* mutant larvae. Both *gbb* mutants and nutrient deprived wild-type larvae show clear decreases in total lipid levels compared to fed wild-type larvae when normalized for either total body mass and/or total protein (Fig. 2A), indicating that they have reduced levels of stored energy. Further analysis using thin layer chromatography (TLC) to separate lipid classes and GC-MS to analyze fatty acid chain length revealed that *gbb* mutant larvae have lower levels of TAGs (Fig. 2B) and significantly reduced levels of short-chain fatty acids (FAs) compared to wild-type larvae (Fig. 2E). Lower levels of short-chain FAs were also observed in wild-type larvae that had been deprived of nutrients for 12 h, as well as in normally fed *Tor* mutant larvae (Figs. 2F, G). *Tor* mutant larvae have been shown previously to have reduced glucose and lipid levels (Luong et al., 2006). And the reduction we observe in short-chain FAs in *Tor* mutants, starved larvae as well as *gbb* mutants, supports the possibility that *gbb* mutant, *Tor* mutant, and fasting larvae could be metabolizing short-chain FAs as a means of obtaining energy (Gutierrez et al., 2007; Kompare and Rizzo, 2008; Reddy and Hashimoto, 2001). Consistent with these changes in nutrient stores observed in *gbb* mutants is the alteration in fat body morphology, specifically the change in lipid droplet size, an alteration that has been suggested to reflect changes in both lipid droplet protein content and modifications in lipid metabolism (Brasaemle, 2007).

gbb mutant larvae mount a starvation response

In principle, depleted nutrient stores in *gbb* mutants may result from a failure of mutant larvae to ingest food. We made use of a fluorescently labeled fatty acid, BODIPY-tagged dodecanoic acid (Bo-C_{12}), mixed into standard Drosophila food to investigate this possibility. If *gbb* mutants are defective in their ability to ingest food, we predicted that they would have less Bo-C_{12} labeled food within their guts. We observed no difference in Bo-C_{12} fluorescence within the lumen of wild-type and *gbb* mutant larval midguts after equivalent feeding regimes (Figs. 3A, B), indicating that *gbb* mutants have no obvious defect in their ability to ingest food. However, in these experiments we observed a difference in Bo-C_{12} fluorescence within the midgut epithelia and fat body lipid

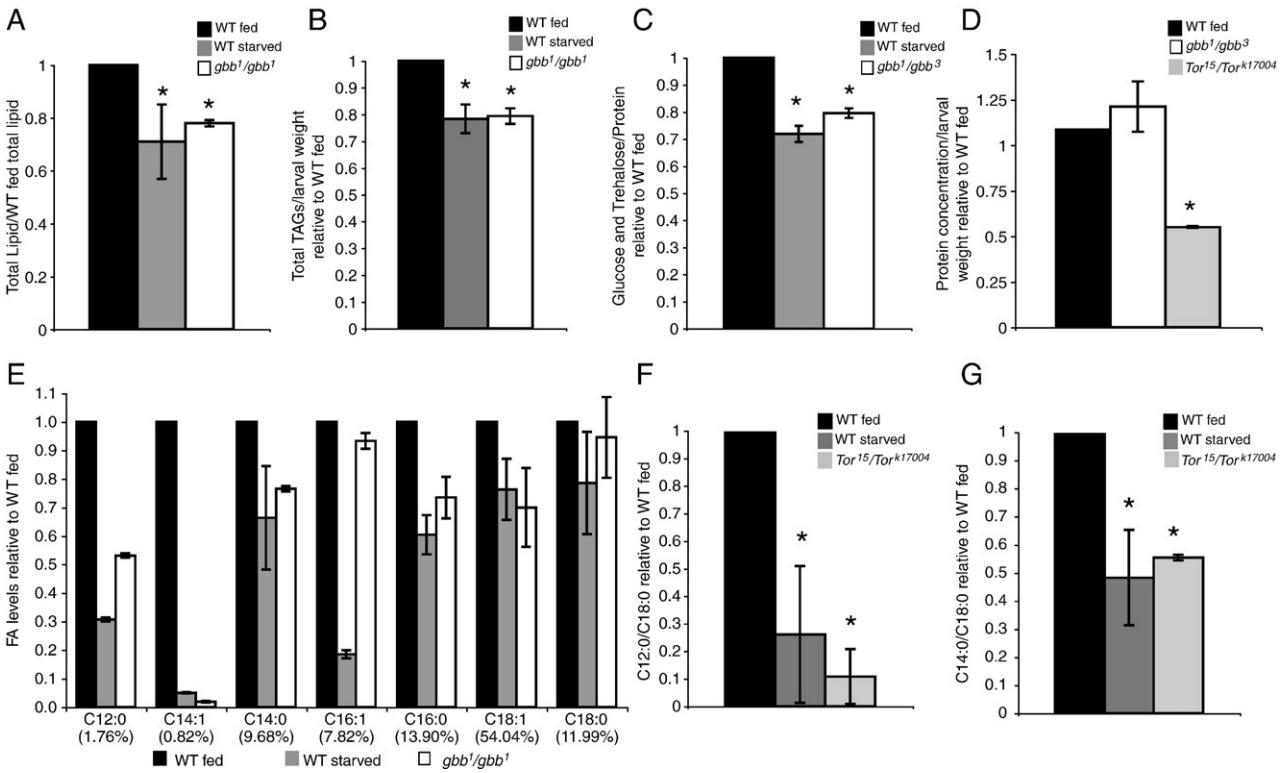

Fig. 2. *gbb* mutant larvae have reduced metabolic stores. (A) Total lipids are reduced in early third instar fed *gbb* null and starved wild-type larvae. (B) After normalization for larval weight, fed *gbb* null larvae and starved wild-type larvae display reduced TAG levels relative to fed wild-type larvae. (C) Glucose and trehalose levels are decreased in wild-type larvae starved for 12 h and *gbb* mutant larvae. *$p<0.05$ compared to wild-type (Student's *t*-test). (D) There is no significant change in the protein:mass ratio in *gbb* mutant larvae compared to wild-type larvae. *Tor* mutant larvae exhibit a reduced protein:mass ratio. (E) GC-MS analysis indicates that early third instar fed *gbb* null and wild-type larvae starved for 12 h have lower levels of short-chain fatty acids compared to wild-type fed larvae. Percent of each fatty acid type in wild-type fed larvae is given in parentheses. (F, G) Short-chain fatty acids are also reduced in *Tor* mutant larvae.

droplets between wild type and *gbb* mutant larvae. When larvae were fed food with 5 μM Bo-C_{12}, *gbb* mutants exhibited significantly higher levels of Bo-C_{12} fluorescence than wild type larvae (Fig. 3, Fig. S2A). This difference in Bo-C_{12} fluorescence did not reflect an inability of Bo-C_{12} to be taken up by cells of the wild type midgut epithelium since dissected midguts simply incubated with Bo-C_{12} readily take it up into their cells (Figure S2C). Furthermore, wild-type larvae raised on food containing higher concentrations of Bo-C_{12} (10 μM) showed high fluorescence within both the midgut epithelium and fat body lipid droplets (data not shown).

Bo-C_{12} is regularly used to monitor fatty acid transport in a number of systems (Tong et al., 2006; Spanier et al., 2009) and given the difference in Bo-C_{12} fluorescence observed between wild type and *gbb* mutant larvae following ingestion of Bo-C_{12}-containing food, we

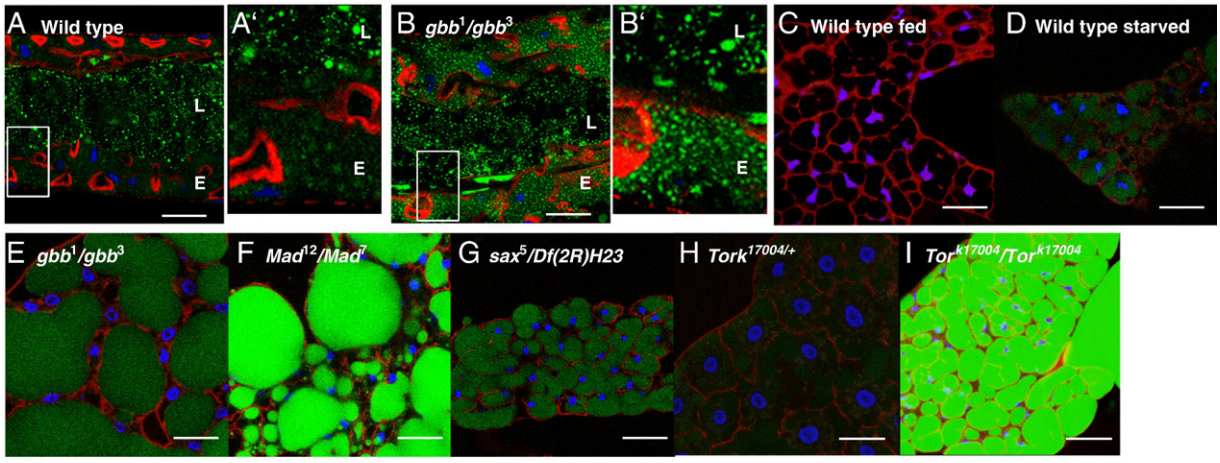

Fig. 3. Loss of BMP signaling leads to alterations in lipid metabolism in the fat body. Midguts and fat bodies from wandering third instar larvae fed Bo-C_{12} (green) and stained with Hoechst (blue) and Phalloidin (red). (A, B) Third instar wild-type (A) and gbb^1/gbb^3 mutant (B) guts ingest Bo-C12 from the food. (A′, B′) Magnification to 300% of regions of the midgut lumen (L) and epithelium (E), indicated by white box, illustrates the increased uptake of Bo-C_{12} by *gbb* mutants in the epithelium. (C) Lipid droplets in fat body cells of wandering third instar wild-type larvae have little Bo-C_{12} fluorescence following continuous feeding of low levels of Bo-C_{12}. (D–I) Wild-type larvae starved for 12 h (D), gbb^1/gbb^3 (E), Mad^{12}/Mad^7 (F), $sax^5/Df(2R)H23$ (G), and $Tor^{k17004}/Tor^{k17004}$ (I) mutant fat bodies exhibit elevated levels of Bo-C_{12} fluorescence within lipid droplets indicative of increased uptake from ingested Bo-C_{12}, likely in response to lower overall metabolic stores (see also text and Fig. S2). Scale bar = 47.62 μm. $n>20$ for each genotype. All images were taken with identical confocal settings.

considered a possible explanation for the apparent difference in fatty acid transport between wild type and gbb mutants. Given that *gbb* mutant larvae have reduced levels of total lipids (Fig. 2A), it is possible that the higher Bo-C_{12} fluorescence observed in *gbb* mutant midgut epithelia and fat bodies reflected an increase in nutrient uptake. An increase in Bo-C_{12} uptake would be consistent with an attempt by the mutant larvae to replenish any reduced lipid stores. Consistent with this interpretation, we found that when we fed Bo-C_{12} food to wild type larvae and then deprived them of any nutrients for 12 h they also displayed a higher level of Bo-C_{12} in their lipid droplets compared to the fat bodies of wild-type larvae continuously fed Bo-C_{12} containing food (Figs. 3C, D). These observations suggest that when food is withdrawn and/or available energy stores are reduced, larvae mount a starvation response by increasing the uptake of available nutrients remaining within the gut lumen, and thus, we detect an increase in Bo-C_{12} uptake and transport from the midgut lumen to the fat body. If the presence of Bo-C_{12} fluorescence in lipid droplets of fasting larvae (Fig. 3D) reflects an increase in the uptake of lipids from the gut, then a pulse of Bo-C_{12} food followed by a chase with standard food, whereby little to no Bo-C_{12} remains in the gut lumen, should result in no difference in lipid droplet fluorescence between fed and starved larvae. Indeed, no difference in the level of fat body Bo-C_{12} fluorescence is seen in these animals (Fig. S2A). Taken together, these results indicate that Bo-C_{12} is readily transported from the midgut to the fat body lipid droplets upon food withdrawal and that the presence of high levels of Bo-C_{12} fluorescence in fat body cells is indicative of animals with altered metabolic status. Consistent with mounting a starvation response, we also found that mutant larvae appear to continually mobilize their stores, as *gbb* mutant (as well as *Mad* mutant, see below) larvae show a decrease in Bo-C_{12} fluorescence despite being supplied with adequate food following feeding on Bo-C_{12}-containing food (Fig. S2A).

Larvae mutant for Gbb/BMP signaling components, *sax* (*sax*^{5}/*Df (2R)H23*) and *Mad* (*Mad*^{12}/*Mad*^{7}), also display high levels of Bo-C_{12} fluorescence in their fat body lipid droplets compared to wild-type larvae (Figs. 3F, G). The increased transport of Bo-C_{12} into fat body lipid droplets that we observed in *gbb* mutant larvae is also apparent in *Mad* mutants. Furthermore, timed feeding experiments with *Mad* mutant larvae show a significant increase in Bo-C_{12} fluorescence by 9 h compared to that seen in wild-type fat bodies (Fig. S2B).

Given that Mad is a downstream transducer for multiple ligands in the Drosophila BMP pathway, we considered the possibility that another BMP ligand, such as the BMP 2/4 orthologue Dpp, may cooperate with Gbb as it does in a number of other developmental processes (Kawase et al., 2004; Khalsa et al., 1998; O'Connor et al., 2006; Shimmi et al., 2005). However, in this case we found that larval-lethal alleles of *dpp* do not exhibit larval transparency or high levels of Bo-C_{12} in fat body droplets (Fig. S3; data not shown), suggesting that Dpp does not influence organismal metabolic status or not to the same extent as Gbb.

Tor mutants are deficient in proper nutrient sensing and show a reduction in lipid stores (Colombani et al., 2003; Luong et al., 2006). Based on our studies with Bo-C_{12} as a probe for fatty acid transport, we tested *Tor* mutants for the level of Bo-C_{12} fluorescence in their fat bodies. Following feeding on Bo-C_{12}-containing food, *Tor* mutants display high levels of fluorescence in their fat body lipid droplets (Figs. 3H, I). This high level of Bo-C_{12} fluorescence is indicative of increased fatty acid transport in response to compromised energy stores and is consistent with the other phenotypic similarities between *Tor* and *gbb* mutant larvae, as well as wild type larvae that have been nutrient deprived.

Gbb/BMP signaling is required in the fat body cells for proper metabolism

The larval fat body is a central player in coordinating the function of more peripheral tissues for Drosophila energy homeostasis (Leopold and Perrimon, 2007). Our data thus far indicate that the fat bodies of *gbb* mutants are morphologically abnormal, they exhibit abnormal transport of the Bo-C_{12} fatty acid, and overall the metabolic stores in *gbb* mutants are not at the level expected of wild type animals. It is possible that the abnormal functioning of the *gbb* mutant fat body results from a loss of Gbb signaling within the fat body itself or a loss from distant tissue(s) that impacts fat body morphology and function. In previous studies, we have shown that *gbb* is broadly expressed the imaginal discs and larval brain (Khalsa et al., 1998). Here, using both *in situ* hybridizations and semi-quantitative RT-PCR on isolated fat bodies, we show that *gbb* is also expressed in the third instar larval fat body (Fig. 4A; data not shown). Furthermore, we find that wild-type fat body cells clearly receive BMP signals as indicated by the high levels of phosphorylated Mad (pMad) localized within their nuclei (Fig. 4C). Wild-type levels of *gbb* are required for this signal, given that little to no pMad accumulates in the nuclei of *gbb* mutant fat body cells (Fig. 4C).

In order to determine whether *gbb* endogenously expressed in fat body cells is required for proper metabolism, we knocked down *gbb* function in the fat body by expressing *gbbRNAi* (*UAS-gbbRNAi*) under the control of the Gal4-UAS system, by using the FB-Gal4 or ppl-Gal4 drivers, each of which have been shown to be expressed in the larval fat body (Colombani et al., 2003; Gronke et al., 2003; Gutierrez et al., 2007). In both crosses, the experimental (e.g. FBGal4>UASgbbRNAi) third instar larvae were transparent, and when raised on food containing Bo-C_{12}, their fat bodies displayed higher Bo-C_{12} fluorescence than those from larvae lacking *UAS-gbbRNAi* (Figs. 4D, E and data not shown). Given that there are no other tissues in common that express both FB-Gal4 and ppl-Gal4 (see Materials and methods), we conclude that a reduction of *gbb* within the fat body is primarily responsible for the obtained phenotypes.

In agreement with a role for *gbb* in the larval fat body, the phenotypes associated with *gbb* mutants including larval transparency, high levels of Bo-C_{12} fluorescence, and lethality are rescued by the expression of wild-type *gbb* in *FBGal4 gbb*^{1}/*UASgbb9.9 gbb*^{1} larvae (Figs. 4B, F; Table S1). A similar rescue of *gbb* mutant phenotypes was achieved with fat body expression of a constitutively active Sax construct (Table S1). Somewhat surprisingly, we found that when BMP signaling is blocked by the overexpression of the inhibitory Smad, Dad (*hsFLP; AyGal4 UAS-nGFP/UAS-dad*), Bo-C_{12} uptake is affected in a cell autonomous manner, as evidenced by the increase in fluorescence in fat body cells overexpressing Dad (overexpressing cells are marked by nuclear GFP) (Fig. 4G). This cell autonomous elevation in lipid droplet Bo-C_{12} fluorescence indicates that the reduction or elimination of BMP signaling within a fat body cell alters the metabolic properties of that cell. Taken together, our findings indicate that Gbb/BMP signaling within the fat body is both necessary and sufficient to maintain normal metabolic physiology.

Finally, we find that nutrient-deprived larvae show a marked reduction or loss of nuclear pMad in their fat body cells (Fig. 4C). This finding indicates that the nutritional status of an animal is not only affected by a reduction in BMP signaling but an external change in nutrient availability can also alter the transduction of BMP signals. Taken together, fat body cells of third instar larvae receive and transduce BMP signals but the metabolic status of these animals both dictates and depends on fat body BMP signaling.

The molecular response to a loss in Gbb signaling is not identical to nutrient deprivation

Drosophila larvae respond to their environment by altering the expression of genes involved in the sensing and storage of nutrients (Zinke et al., 2002). A distinct change in the transcript level of genes that control the breakdown of stored lipids and proteins is seen in Drosophila larvae deprived of nutrients (Zinke et al., 1999, 2002). We compared the expression levels of several of these starvation-

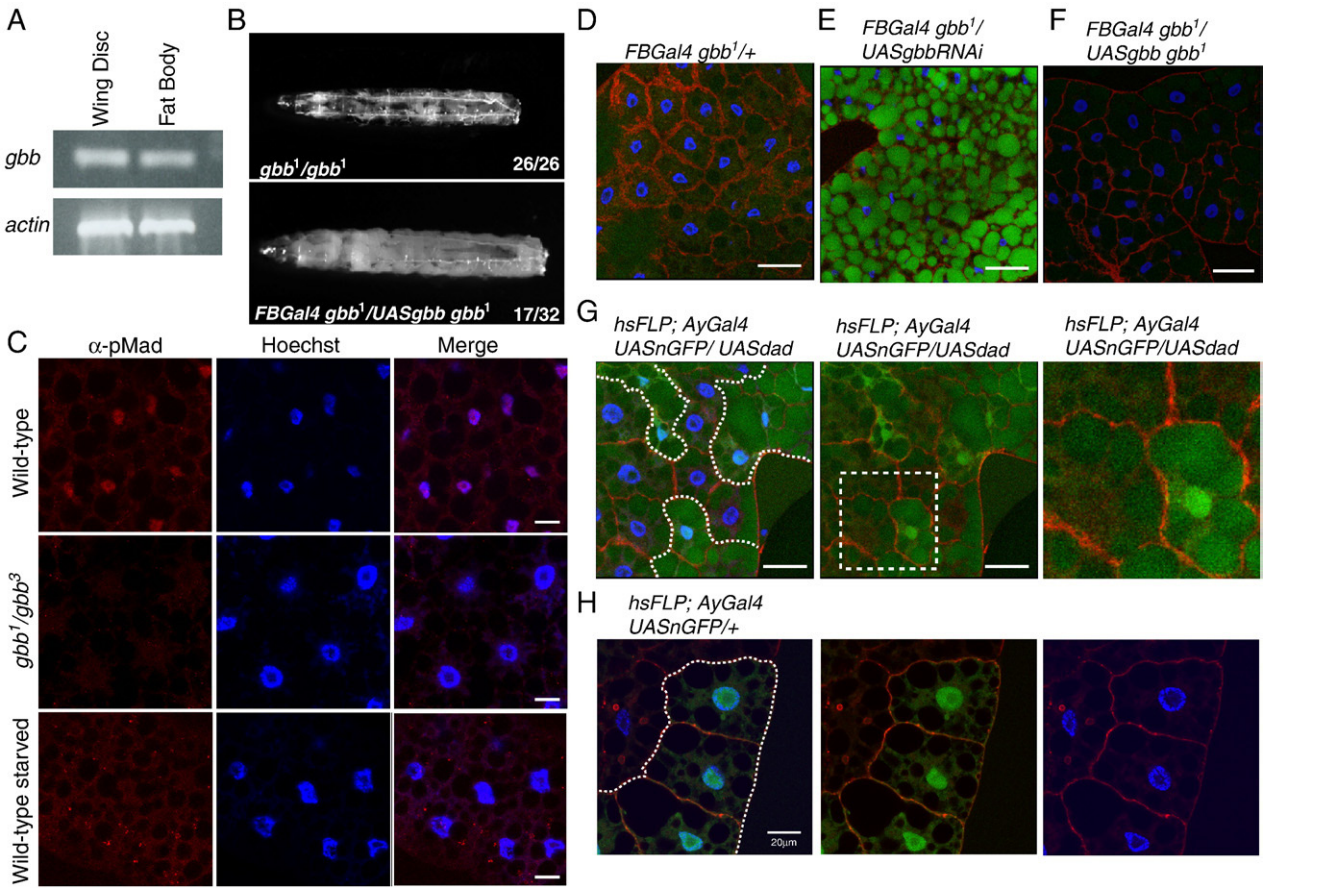

Fig. 4. BMP signaling in the fat body is required for proper lipid metabolism. (A) RT-PCR shows that *gbb* mRNA is expressed in isolated larval fat body tissue and wing imaginal discs, a tissue where *gbb* expression has previously been established (Khalsa et al., 1998). (B) The clarity of 47% of *gbb* null larvae is rescued by expression of *gbb* in the fat body (*FBGal4 gbb^1/UASgbb9.9 gbb^1*). The number of transparent larvae observed is given as a ratio of total larvae examined. *p<0.01 (Chi square analysis compared to *gbb^1/gbb^1*). (C) pMad accumulates in the nuclei of wild-type fat bodies (top row) but not in *gbb^1/gbb^3* mutant fat bodies (middle row) or in approximately 65% of fat bodies from wild-type larvae deprived of nutrients for 12 h (bottom row). n>12. Scale bar = 20 μm. (D, E) Knockdown of endogenous *gbb* expression in the fat body by RNAi leads to an increase in Bo-C_{12} fluorescence (green) (E) compared to control fat bodies (D). (F) Fat body expression of *gbb* in *gbb* null larvae that were fed Bo-C_{12} restores proper lipid metabolism to little to no Bo-C_{12} fluorescence levels. (G) FLPout clones (outlined by dotted white line in left panel and marked by the presence of nuclear GFP) overexpressing *dad* produce higher levels of Bo-C_{12} fluorescence in lipid droplets. Right panel shows high mag image of boxed area in middle panel. Scale bar = 47.62 μm. (H) Control FLPout clone (outlined by dotted white line and marked by the presence of nuclear GFP) in third instar larvae not fed Bo-C_{12} food shows lack of GFP clone marker in lipid droplets. Scale bar = 20 μm.

responsive genes between wild type larvae both fed and deprived of nutrients, and from *gbb* mutant larvae, also both fed and nutrient deprived (Fig. 5). In both fed *gbb* mutants and starved wild-type larvae, a lower level of expression level is seen for the amino acid transporter encoded by *minidiscs* (*mnd*), which is reportedly expressed exclusively in the fat body (Martin et al., 2000) (Fig. 5A). In contrast, an increase is seen in the transcript levels of *lipase3* (*lip3*), whose expression appears to be limited to the larval fat body (Pistillo et al., 1998), and Drosophila insulin-like peptide 2 (*dilp2*), whose expression is abundant in symmetrical clusters of neurons in the larval brain and in the larval salivary glands and at low levels in the imaginal discs (Brogiolo et al., 2001) (Fig. 5B and F). Interestingly, other starvation-responsive genes, such as the human adipocyte triglyceride lipase (ATGL) orthologue, encoded by *brummer* (*bmm*) and a human acid lipase orthologue encoded by *lipase4* (*Lip4/CG6113*) display a significant change in expression levels in wild-type starved larvae but not in fed *gbb* mutant larvae (Fig. 5E; data not shown). *Brummer* is expressed at all stages of development and its larval expression is limited to the fat body, midgut, and gastric caeca (Gronke et al., 2005). In addition, the proposed long chain fatty acid transporter (*CG4563*) and the fatty acid synthase BcDNA:GH07626 display an expected increase and decrease in mRNA levels, respectively, in wild-type starved larvae; however, there is no change in their level of expression in fed *gbb* mutants (data not shown). The expression patterns of *lip4*, *CG4563*, and the fatty acid synthase BcDNA:GH07626 have not yet been determined. Taken together these results indicate that *gbb* mutants do not precisely mimic the transcriptional response induced by nutrient deprivation.

Despite not mimicking a starvation response at the transcriptional level, *gbb* mutants could suffer from a reduced amino acid uptake through the permease-like Mnd transporter and from alterations in lipid and sugar metabolism due to increased levels of *lipase 3* and *dilp2* (Zinke et al., 1999, 2002). However, because our data to this point identified some similarity between *gbb* mutants and nutrient deprived animals with regards to metabolic stores, etc., we next asked if *gbb* mutants are able to properly mount a starvation response. We find that when deprived of nutrients, *gbb* mutants show the expected increase (*lip3, bmm,* and *dilp2*) or decrease (*mnd*) of gene expression that is seen in nutrient deprived wild-type larvae (Figs. 5A, B, E and F). Furthermore, nutrient deprived *gbb* mutants show an increase in *CG4563* and a decrease in *BcDNA:GH07626* expression levels consistent with their ability to mount a starvation response (data not shown). Thus, the metabolic changes observed in *gbb* mutants result in a change in the expression of some genes involved in nutrient processing but not all.

Upon nutrient deprivation wild-type larvae do not display a significant change in the level of transcription of some genes required for lipid transport, such as, *Retinoid and fatty-acid binding protein* (*RfaBp*), the vertebrate apolipoprotein 1 and 2 orthologue, and *CG31217*, a low-density lipoprotein (LDL) receptor (Zinke et al.,

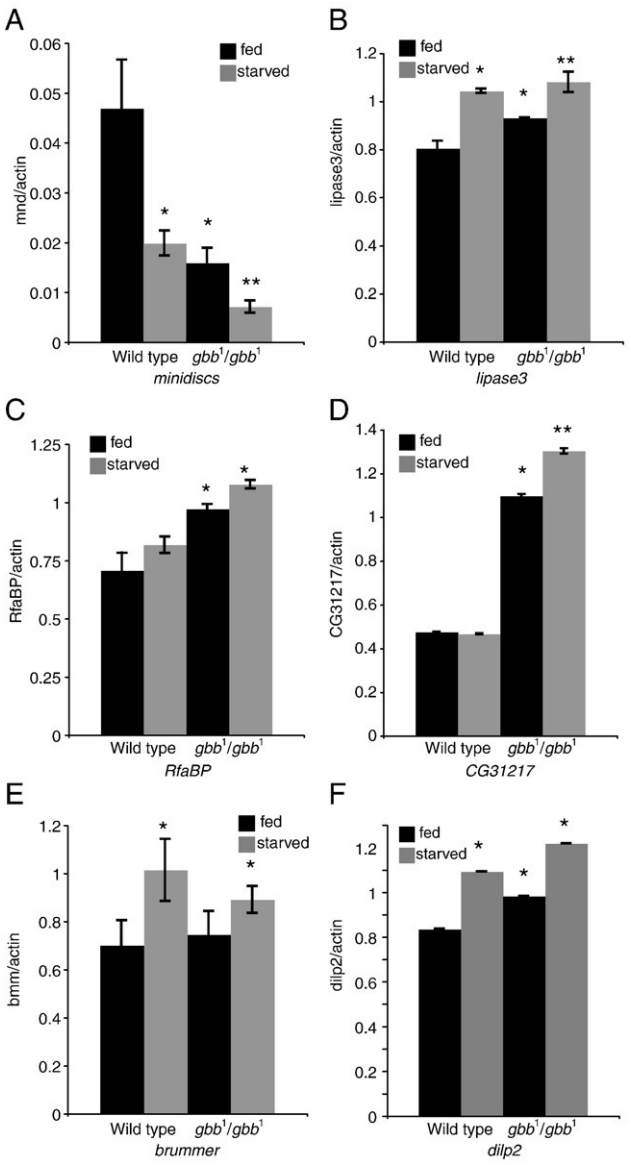

Fig. 5. gbb mutants show alteration in the expression of some starvation-responsive genes. (A) *mnd* expression in early third instar larvae is reduced in wild-type larvae deprived of nutrients for 12 h as well as in fed *gbb* null larvae. A further reduction in transcript levels occurs when *gbb* null larvae are starved for 12 h. (B) *lip3* expression is increased in starved wild-type larvae and in fed *gbb* null larvae. Depriving *gbb* mutants of nutrients further increases the elevated *lip3* mRNA levels. (C) *RfaBp* mRNA levels are elevated in fed and starved *gbb* null larvae but are not altered in nutrient-deprived wild-type larvae. (D) *CG31217* (low density LDL receptor) expression is elevated in fed and starved *gbb* mutants relative to wild-type fed larvae. (E) *bmm* lipase expression does not increase in *gbb* mutants as in wild-type starved larvae. However, *bmm* transcript levels slightly increase in starved *gbb* mutants. (F) *dilp2* mRNA levels are elevated in starved wild-type larvae as well as fed and starved *gbb* mutant larvae. In each RT-PCR sample band intensity of gene is normalized to band intensity of actin. $*p<0.05$ compared to wild-type fed larvae; $**p<0.05$ compared to wild-type and gbb^1/gbb^1 fed larvae (Student's *t*-test).

2002). However, we find that transcript levels for both of these genes are increased in *gbb* mutants (Figs. 5C, D), further highlighting the difference between the organismal responses to a loss of Gbb signaling versus the deprivation of nutrients. Another interesting difference between *gbb* mutant larvae and nutrient-deprived larvae is in the total level of C16:1 fatty acids present in third instar larvae. *gbb* mutants show similar levels of C16:1 FA as wild type fed larvae and thus, do not seem to metabolize these fatty acids as nutrient-deprived larvae (Fig. 2E). Thus, while *gbb* mutants exhibit many morphological and physiological similarities to nutrient-deprived larvae, the molecular response to reduced Gbb signaling is different from that observed when nutrient availability is reduced.

In line with this conclusion, we find that while the oenocytes of wild-type larvae accumulate lipids during periods of nutrient deprivation (Gutierrez et al., 2007), the oenocytes of fed *gbb* mutants do not show an accumulation of neutral lipids (Fig. S4). Upon starvation, the oenocytes of *gbb* mutant larvae accumulate neutral lipids, consistent with the ability of *gbb* mutants to elicit a starvation response.

Discussion

The nutritional status of an organism dictates its growth and maturation during development. How the balance of nutrient uptake, storage, and metabolism is coordinated with growth and developmental progression is not thoroughly understood. More recently, specific genetic and physiological pathways responsible for the coordination of growth, development and metabolism are being identified in a number of metazoan systems (Edgar, 2006; Leopold and Perrimon, 2007; Saltiel and Kahn, 2001). Here, we show that the Gbb/BMP signaling pathway regulates the metabolic status of Drosophila larvae, in addition to its role in cell fate specification. Previous studies have shown that BMP2 and BMP4, as well as a BMP transcriptional cofactor, Schnurri, can influence adipocyte differentiation (Jin et al., 2006) and that BMPs can promote the accumulation of lipids in mammalian brown pre-adipocytes (Tseng et al., 2008). However, the data presented here indicate that BMP signaling can also have a significant impact on nutrient uptake and metabolic status of the organism well after fat cell differentiation, during its progression through larval development.

gbb mutant larvae resemble nutrient deprived larvae in that they are transparent, exhibit growth defects, and utilize stored energy sources, especially short chain fatty acids (Figs. 1, 2). When deprived of food, wild-type larvae mount a starvation response that results in the uptake of nutrients remaining in the gut, as indicated by Bo-C_{12} pulse-chase experiments. Interestingly, *gbb* mutants show a higher level of Bo-C_{12} uptake despite the fact that they are not deprived of food. This indicates that while *gbb* mutants ingest food and take up nutrients, they are unable to maintain energy stores and thus, share physiological similarities with starved larvae. However, despite physiological and morphological similarities, nutrient-deprived wild-type larvae and fed *gbb* mutant larvae exhibit somewhat different gene expression profiles, based on the transcript levels of several starvation-responsive genes (Fig. 5). Such dissimilarities in molecular responses suggest fundamental differences in the mechanisms responsible for "environmental starvation" and "genetically-induced *gbb* mutant starvation."

Larval transparency is perhaps the most striking aspect of the "starvation" phenotype, and changes in the appearance of the larval fat body are largely responsible for this phenotype. Given that all regions of the fat body are present in *gbb* mutant animals, albeit exhibiting smaller cells with lipid droplets of varying sizes, and especially since these defects can be rescued by the expression of wild type gbb in the larval period, there is no indication that *gbb* mutants are defective in the specification and differentiation of the fat body in general, or in specific regions of this organ. Furthermore, defects in *gbb* mutant larvae are not evident until the late second/early third larval instar (S. Ballard and K. Wharton, personal observation). Thus, the role for Gbb signaling appears to be a later function that influences metabolism, and not one in the specification of a fat body fate or in the specific differentiation of fat body cells.

The fat body tissue is the central metabolic organ within the Drosophila larva that communicates the nutritional status of the organism to other tissues to influence their growth and function. At the same time, various tissues communicate to the fat body to promote proper storage and mobilization of energy. The defects in

both the morphology and function of the fat body in *gbb* mutants can be at least in part accounted for by a loss or reduction in BMP signaling within the fat body itself. *gbb* is expressed in the fat body, and *gbb* is required for the nuclear localization of pMad within these cells (Fig. 4), suggesting that Gbb acts within the fat body to provide active BMP signaling. A loss of Gbb/BMP signaling due to the expression of either *gbb-RNAi* or *dad*, within fat body cells compromises the nutritional status of the fat body in an autonomous manner. Expression of wild type *gbb* within the fat body can partially rescue larval transparency, increased levels of Bo-C_{12} fluorescence, and lethality caused by loss of Gbb signaling (Figs. 4B and F; Table S1). Not all *gbb* mutant phenotypes are rescued when *gbb* is expressed in the fat body. The synapses at the larval neuromuscular junction (NMJ) are reduced in size in *gbb* mutants, with a decrease in overall bouton number (McCabe et al., 2003 and Fig. S6). This undergrowth is not rescued when *gbb* is expressed in the fat body (Fig. S6). In addition, the patterning defects observed in the adult *gbb* mutant wing were not rescued (S. Ballard and K. Wharton, personal observation). Furthermore, we do not find that when *gbb* expression is increased in the fat body of wild type larvae (*FB-Gal4>UASgbb*) that a concomitant increase is seen in the size of wing discs (Fig. S5), an effect we observe when *gbb* is specifically expressed in the wing imaginal disc (Khalsa et al., 1998). If Gbb were solely influencing cell/tissue growth through secretion into, and circulation via, the hemolymph, then we would expect increased expression of Gbb from tissues such as the fat body to influence distant tissues, such as the NMJ and the growth of imaginal discs. This is not what is observed, as discs from *FB-Gal4/UASgbb* larvae are comparable in size to wild-type discs.

However, we do find that the reduced size of *gbb* mutant wing imaginal discs can be partially rescued by *gbb* expression within the fat body (Fig. S5). While it is possible, albeit unlikely as discussed above, that elevated levels of Gbb secreted into the hemolymph could be responsible for rescue, our results more strongly support the likelihood that the role of Gbb signaling in regulating metabolism via its effect on the physiology of fat body cells is rescued and thus, nutrients and energy are available for the growth of distant tissues. Our results point to an essential role for Gbb/BMP signaling within the fat body itself for regulating metabolism, however, they do not completely rule out the possibility that Gbb/BMP could also signal from another defined site to impact fat body function.

The hepatocyte-like oenocytes have been implicated in lipid metabolism based on their starvation induced accumulation of lipids following fat body depletion of lipid stores (Gutierrez et al., 2007). In *gbb* mutants, we did not detect an accumulation of lipids in the oenocytes as seen for starved wild-type animals which could have suggested that loss of Gbb signaling affected the communication between oenocytes and the fat body. Rather, we found that when *gbb* mutants were deprived of nutrients they showed an accumulation of neutral lipids, albeit not at the level of wild-type larvae, presumably because *gbb* mutants have lower total triglycerides than wild-type (Fig. S4). We did not find that when a potential function of *gbb* in oenocytes was disrupted by the expression of *gbb-RNAi* by BO-Gal4 (Gutierrez et al., 2007) (*BO-Gal4 gbb^{1}/UASgbbRNAi*) that there was any effect on the number of oenocytes or oenocyte clusters. Nor did we find that this manipulation induced a transparent larval phenotype or an increase of Bo-C_{12} in fat body lipid droplets (data not shown). Finally, we did not observe nuclear pMad in oenocytes nor a change in pMad distribution in *gbb* mutant or nutrient deprived larvae. Thus, the loss of *gbb* function does not appear to impact the specification or function of oenocytes in lipid uptake.

The larval brain is another important player in maintaining energy homeostasis and it communicates with the fat body through the secretion of insulin-like peptides (*dilps*) and adipokinetic hormone, AKH (glucagon), to either promote storage of nutrients or the breakdown of metabolic stores, respectively (Ikeya et al., 2002; Lee and Park, 2004; Van der Horst, 2003). We found that *gbb* mutant larvae have elevated levels of *dilp2* expression but despite these changes we were unable to detect a synergistic genetic interaction between mutations in Gbb signaling and the insulin receptor (*InR*) or the insulin receptor substrate *chico* (M. Psotka, K. Wharton, unpublished). It is of interest, however, that an increase in insulin is associated with a mobilization of stored carbohydrates. In Drosophila, Broughton et al. (2007) have shown that a specific decrease in *dilp2* expression within the mNSCs (medial neurosecretory cells) of the brain results in increased levels of whole body trehalose, and interestingly, in decreased levels of *dilp-3* and *-5*. Furthermore, it is interesting to note that profound changes in foraging behavior is associated with animals deprived of nutrients (Sokolowski, 2001), and recent work has shown that these behaviors are elicited by elevated levels of *dilp2* and *dilp4* (Wu et al., 2005). Consistent with these findings and the increased level of *dilp2* expression observed in *gbb* mutants, we have observed that late third instar *gbb* mutant larvae forage for long periods (data not shown).

The balance between energy stored and energy expended is critical for animal growth and survival. Our results have clearly shown that a reduction in Gbb/BMP signaling impacts nutrient stores and metabolism and accordingly, we observe an up-regulation of some "starvation-responsive" genes, indicating that Gbb/BMP signaling acts to promote nutrient storage. Interestingly, when animals are deprived of an external source of nutrients, we in turn observe a loss of BMP signaling (pMad) in fat body cells, consistent with the organism's need to mobilize nutrients and to postpone nutrient storage (Fig. 6). The TOR pathway has been shown to be an important player in metabolism and *Tor* also appears to act in the Drosophila fat body. The similarities in larval phenotypes between Gbb/BMP mutants and TOR pathway mutants are striking. The fact that *Tor* mutants also show lower levels of short chain FAs and total lipids as well as differences in lipid transport is consistent with a central role for TOR in nutrient sensing and homeostasis. Interestingly, we find that the phenotypes of Gbb/BMP pathway mutants are affected by alterations in TOR function and vice versa (S. Ballard and K. Wharton, unpublished). Our preliminary data

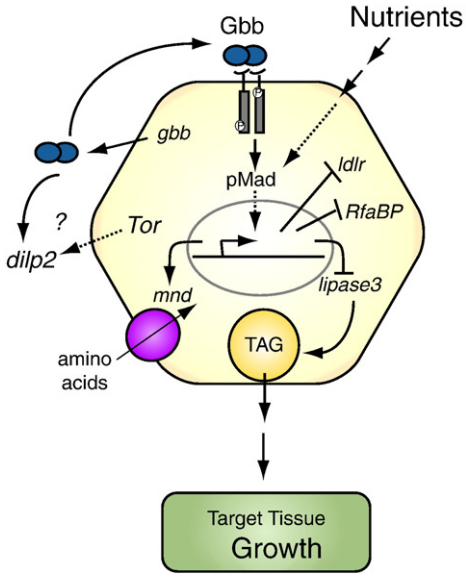

Fig. 6. Gbb/BMP signaling regulates energy homeostasis. The model schematizes the proposed action of Gbb/BMP signaling in the fat body to regulate energy homeostasis. *gbb* is expressed in fat body cells and Gbb/BMP signaling is active within this tissue through the phosphorylation and translocation of pMad to the nucleus. Gbb signaling influences the transcription of genes involved in amino acid uptake (*mnd*), sugar metabolism (*dilp2*), and lipid hydrolysis (*lip3*) and transport (*RfaBp* and *CG31217* (LDL receptor)). Interestingly, in addition to its role in promoting nutrient storage BMP signaling is itself responsive to the level of metabolic stores. When nutrient levels are low the accumulation of pMad in fat body nuclei is lost.

indicate that there is a feedback mechanism in place involving these two pathways such that the organism is able to achieve homeostasis. While the specifics of the molecular mechanisms underlying this feedback await further characterization, our observations have revealed an intimate relationship between BMP signaling and a developing organism's metabolic status, whereby Gbb/BMP signaling is critical for attaining energy homeostasis with BMP signals likely acting to influence the fat body's ability to coordinate nutrient uptake, storage, and energy availability with tissue growth. Further studies that uncover the molecular details of how BMP signaling impacts the balance of energy storage and mobilization will be critical for tackling many diseases, not only obesity and metabolic syndromes, but those that affect other tissues such as neural (Huntington's, Parkinson's, Alzheimer's, Tuberous sclerosis) and bone (fibrodysplasia ossificans progressiva) (Baggio, 2002; Farooqui et al., 2007; Shore et al., 2006) where it is clear that an individual's metabolic state impacts the presentation and progression of disease.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ydbio.2009.11.011.

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